

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**



(19) Europäisches Patentamt
European Patent Office
Office européen des brevets



(11) EP 0 935 001 A1

(12) **EUROPEAN PATENT APPLICATION**
published in accordance with Art. 158(3) EPC

(43) Date of publication:
11.08.1999 Bulletin 1999/32

(51) Int. Cl.⁶: C12N 15/18, C12N 15/63,
C12P 21/02, C07K 14/485,
C07K 16/22, G01N 33/50

(21) Application number: 97930804.6

(22) Date of filing: 15.07.1997

(86) International application number:
PCT/JP97/02456

(87) International publication number:
WO 98/02543 (22.01.1998 Gazette 1998/03)

(84) Designated Contracting States:
AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC
NL PT SE

(72) Inventors:
• HIRATA, Yulchi,
Chugai Research Institute
Niihari-gun, Ibaraki 300-41 (JP)
• NEZU, Junichi,
Chugai Research Institute
Niihari-gun, Ibaraki 300-41 (JP)

(30) Priority: 15.07.1996 JP 18521696

(74) Representative:
VOSSIUS & PARTNER
Postfach 86 07 67
81634 München (DE)

(71) Applicant:
Chugai Research Institute for Molecular
Medicine Inc.
Niihari-gun, Ibaraki 300-41 (JP)

(54) **NOVEL VEGF-LIKE FACTORS**

(57) A novel human gene having a significant homology with a VEGF-C gene, a member of the VEGF family, has been isolated by the PCR method using primers designed based on the sequence of EST that is assumed to be homologous with the C-terminal region of the VEGF-C gene. Mouse and rat genes have been isolated based on the human gene isolated as above. A protein encoded by the above human gene has been isolated by introducing the gene into *Escherichia coli* and expressing it. The isolated protein and genes can be applied to, for example, gene therapy for the VEGF-D deficiency, wound healing, and promotion of collateral vessel formation. Furthermore, VEGF-D protein inhibitors can be used as a novel anticancer drug, etc.

EP 0 935 001 A1

Description

Technical Field

5 [0001] The present invention relates to a protein factor involved in angiogenesis in humans and falls in the field of genetic engineering.

Background Art

10 [0002] The process of angiogenesis, in which endothelial cells existing in the inner wall of blood vessels of animals generate new blood vessels, is triggered by transduction of a specific signal. A variety of substances are reportedly involved in this signal transduction. The most notable substance among them is the vascular endothelial growth factor (VEGF). VEGF is a protein factor which was isolated and purified, and can increase the proliferation of endothelial cells and the permeability of blood vessels (Senger, D. R. et al., *Science* 219: 983-985 (1983); Ferrara, N. and Henzel, W. J., *Biochem. Biophys. Res. Commun.* 161: 851-858 (1989)). It has been reported that the human VEGF gene contains

15 eight exons and produces four subtypes consisting of 121, 165, 189, or 206 amino acid residues, depending on the difference in splicing, which causes different secretion patterns (Houck, K. A. et al., *Mol. Endocrinol.* 5: 1806-1814 (1991)). It has also been reported that there is a VEGF-specific receptor, fit-1, and that the binding of VEGF to fit-1 is important for the signal transduction (Vries, C. D. et al., *Science* 255: 989-991 (1992)).

20 [0003] Placental growth factor (PIGF) and platelet-derived growth factor (PDGF) have thus far been isolated and are factors related to VEGF. These factors are found to promote proliferation activities of vascular endothelial cells (Maglione, D. et al., *Proc. Natl. Acad. Sci. USA* 88: 9267-9271 (1991); Betsholtz, C. et al., *Nature* 320: 695-699 (1986)). In addition, VEGF-B (Olofsson, B. et al., *Proc. Natl. Acad. Sci. USA* 93: 2576-2581 (1996)) and VEGF-C (Lee, J. et al., *Proc. Natl. Acad. Sci. USA* 93: 1988-1992 (1996); Joukov, V. et al., *EMBO J.* 15, 290-299 (1996)) have recently been isolated.

25 [0004] These factors appear to constitute a family, and this may contain additional unknown factors.

[0005] It has been suggested that VEGF is involved in not only vascular formation at the developmental stage but also in the pathological neovascularization associated with diabetes, rheumatoid arthritis, retinopathy, and the growth of solid tumors. Furthermore, in addition to its vascular endothelial cell growth-promoting effects listed above, VEGF's ability to increase vascular permeability was suggested to be involved in the edema formation resulting from various causes. Also, these VEGF family factors may act on not only the blood vessels but also the blood cells and the lymphatic vessels. They may thus play a role in the differentiation and proliferation of blood cells and the formation of lymphatic vessels. Consequently, the VEGF family factors are presently drawing extraordinary attention for developing useful, novel drugs.

35 **Disclosure of the Invention**

[0006] An objective of the present invention is to isolate a novel protein belonging to the VEGF family and a gene encoding the protein. We searched for genes having homology to VEGF-C, which is a recently cloned VEGF family gene, against Expressed Sequence Tags (EST) and Sequence Tagged Sites (STS) in the GenBank database. As a result, we found an EST that was assumed to have homology to the C-terminal portion of VEGF-C. We then designed primers based on the sequence, and amplified and isolated the corresponding cDNA using the 5' RACE method and the 3' RACE method. The nucleotide sequence of the isolated cDNA was determined, and the deduced amino acid sequence therefrom revealed that the amino acid sequence had significant homology to that of VEGF-C. Based on the homology, we have assumed that the isolated human clone is a fourth member of the VEGF family (hereinafter designated as VEGF-D). We have also succeeded in expressing the protein encoded by the isolated human VEGF-D gene in *E. coli* cells, and have also purified and isolated it. Furthermore, we have succeeded in isolating the mouse and rat VEGF-D genes using the isolated human VEGF-D gene.

[0007] In particular, the present invention relates to a novel protein belonging to the VEGF family and a gene encoding the protein. More specifically it relates to

- (1) A protein shown by SEQ ID NO.1 or having the amino acid sequence derived therefrom in which one or more amino acids are substituted, deleted, or added;
- (2) A protein encoded by a DNA that hybridizes with the DNA shown by SEQ ID NO. 2;
- (3) A DNA encoding the protein of (1);
- (4) A DNA hybridizing with the DNA shown by SEQ ID NO. 2;
- (5) A vector containing the DNA of (3) or (4);
- (6) A transformant carrying the vector of (5);

- (7) A method of producing the protein of (1) or (2), which comprises culturing the transformant of (6);
- (8) An antibody binding to the protein of (1) or (2);
- (9) A method of screening a compound binding to the protein of (1) or (2), which comprises a step of detecting the activity of the protein of (1) or (2) to bind to a test sample; and
- 5 (10) A compound binding to the protein of (1) or (2), wherein said compound has been isolated by the method of (9).

[0008] The protein of the present invention (VEGF-D) has significant homology to VEGF-C and can be considered to be a fourth factor of the VEGF family. Since the major function of VEGF is vascular formation at the developmental 10 stage and VEGF is considered to be involved in the pathological neovascularization associated with diabetes, rheumatoid arthritis, retinopathy, and the growth of solid tumors, the protein of the present invention is thought to have similar functions.

[0009] A person skilled in the art could prepare functionally equivalent proteins through modifying VEGF-D of the present invention by adding, deleting, or substituting one or more of the amino acids of VEGF-D shown by SEQ ID NO. 15 1 using known methods. Modifications of the protein can also occur naturally in addition to the artificial modifications described above. These modified proteins are also included in the present invention. Known methods for adding, deleting, or substituting amino acids include the overlap extension polymerase chain reaction (OE-PCR) method (Gene, 1989, 77 (1): 51).

[0010] The DNA encoding VEGF-D of the present invention, shown by SEQ ID NO. 2, is useful for isolating DNAs 20 encoding the proteins having similar functions to VEGF-D in other organisms. For example, a person skilled in the art could routinely isolate homologs of human VEGF-D of the present invention from other organisms by allowing the DNA shown by SEQ ID NO. 2, or part thereof, as a probe, to hybridize with the DNA derived from other organisms. The DNA that hybridizes with the DNA shown by SEQ ID NO. 2 is also included in the present invention. The other organisms include mice, rats, and rabbits.

[0011] The DNA encoding a protein that is functionally equivalent to VEGF-D usually has high homology to the DNA 25 shown by SEQ ID NO. 2. The high homology used herein means at least 70% or higher, more preferably 80% or higher, and still more preferably 90% or higher of sequence homology.

[0012] An example of the hybridization conditions for isolating the DNA having high homology will be given below. Prehybridization is performed in ExpressHyb Solution at 68°C for 30 minutes. The probe labeled with a radioisotope is 30 denatured at 95°C to 100°C for 2 to 5 minutes and rapidly chilled on ice. The probe is added to a new ExpressHyb Solution. The blot is transferred to the solution containing the probe and allowed to hybridize under a temperature gradient of 68°C to 55°C for 2 hours. The blot is washed four times, for 10 minute each, with a 2 x SSC solution containing 0.05% SDS at room temperature. The blot is then washed with a 0.1 x SSC solution containing 0.1% SDS at 45°C for 3 minutes. The blot is subjected to autoradiography.

[0013] An example of the hybridization conditions for isolating the DNA having very high homology will be given below. Prehybridization is performed in ExpressHyb Solution at 68°C or 30 minutes. The probe labeled with a radioisotope is 35 denatured at 95°C to 100°C for 2 to 5 minutes and rapidly chilled on ice. The probe is added into a new ExpressHyb Solution. The blot is transferred into the solution containing the probe, and allowed to hybridize at 68°C for 1 hour. The blot was washed four times, for 10 minute each, with a 2 x SSC solution containing 0.05% SDS at room temperature. The blot was then washed with a 0.1 X SSC solution containing 0.1% SDS at 50°C for 40 minutes, during which the solution was replaced once. The blot was then subjected to autoradiography.

[0014] Note that the hybridization condition can vary depending on the length of the probe (whether it is an oligomer or a probe with more than several hundred bases), the labeling method (whether the probe is radioisotopically labeled or non-radioisotopically labeled), and the type of the target gene to be cloned. A person skilled in the art would properly 40 select the suitable hybridization conditions. In the present invention, it is especially desirable that the condition does not allow the probe to hybridize with the DNA encoding VEGF-C.

[0015] The DNA of the present invention is also used to produce VEGF-D of the present invention as a recombinant 45 protein. Specifically, the recombinant protein can be produced in large quantity by incorporating the DNA encoding VEGF-D (for example, the DNA shown by SEQ ID NO. 2) into a suitable expression vector, introducing the resulting vector into a host, and culturing the transformant to allow the recombinant protein to be expressed.

[0016] The vector to be used for producing the recombinant protein is not particularly restricted. However, vectors such as pGEMEX-1 (Promega) or pEF-BOS (Nucleic Acids Res. 1990, 18(17): p.5322) are preferable. Suitable examples of the host into which the vector is introduced include E. coli cells, CHO cells, and COS cells.

[0017] The VEGF-D protein expressed by the transformant can be purified by suitably combining purification treatments such as solubilization with a homogenizer or a sonicator, extraction by various buffers, solubilization or precipitation by acid or alkali, extraction or precipitation with organic solvents, salting out by ammonium sulfate and other agents, dialysis, ultrafiltration using membrane filters, gel filtration, ion exchange chromatography, reversed-phase chromatography, counter-current distribution chromatography, high-performance liquid chromatography, isoelectric 55

focusing, gel electrophoresis, or affinity chromatography in which antibodies or receptors are immobilized.

[0018] Once the recombinant protein is obtained, antibodies against it can be prepared using known methods. The known methods include preparing polyclonal antibodies by immunizing rabbits, sheep, or other animals with the purified protein, and preparing monoclonal antibodies from the antibody-producing cells of immunized mice or rats. These antibodies will make it possible to quantify VEGF. Although the antibodies thus obtained can be used as they are, it will be more effective to use the humanized antibodies to reduce the immunogenicity. The methods of humanizing the antibodies include the CDR graft method and the method of directly producing a human antibody. In the CDR Graft method, the antibody gene is cloned from the monoclonal antibody-producing cells and its antigenic determinant portion is transplanted into an existing human antibody. In the method of directly producing a human antibody, a mouse whose immune system has been replaced by the human immune system is immunized, similar to ordinary monoclonal antibodies. The VEGF-D protein or its antibody thus obtained can be administered into the body by subcutaneous injection or a similar method.

[0019] A person skilled in the art could screen compounds that bind to the protein of the present invention by known methods.

[0020] For example, such compounds can be obtained by making a cDNA library on a phage vector (such as λgt11 and ZAP) from the cells expected to express the protein that binds to the protein of the present invention (such as lung, small intestine, and heart cells of mammals), expressing the cDNAs on LB-agarose, fixing the expressed proteins onto a filter, preparing the purified protein of the present invention as a biotin-labeled or a fusion protein with the GST protein, and reacting this protein with the above filter. The desired compounds could then be detected by west western blotting using streptavidin or an anti-GST antibody (Skolnik, E. Y., Margolis, B., Mohammadi, M., Lowenstein, E., Fischer, R., Drepps, A., Ullrich, A., and Schlessinger, J. (1991) Cloning of P13 kinase-associated p85 utilizing a novel method for expression/cloning of target proteins for receptor tyrosine kinases, *Cell* 65: 83-90). Another method comprises the following steps. First, express the protein of the present invention fused with the SRF binding domain or the GAL4 binding domain in yeast cells. Second, prepare a cDNA library which expresses cDNAs fused with the transcription activation domain of VP16 or GAL4 from the cells expected to express a protein that binds to the protein of the present invention. Third, introduce the cDNA into the above yeast cells. Fourth, isolate the library-derived cDNA from the positive clones. Finally, introduce the isolated cDNA into *E. coli* to allow it to be expressed. (When a protein that binds to the protein of the present invention is expressed in yeast cells, the reporter gene is activated and the positive clone can be detected.) This method can be performed using the two-hybrid system (MATCHMAKER Two-Hybrid system, Mammalian MATCHMAKER Two-Hybrid Assay Kit, or MATCHMAKER One-Hybrid System (all by Clontech) or the HybriZAP Two-Hybrid Vector System (Stratagene) (Dalton, S. and Treisman, R. (1992) Characterization of SAP-1, a protein recruited by serum response factor to the c-fos serum response element, *Cell* 68: 597-612). Alternatively, the binding proteins can be screened by preparing a cDNA library from the cells expected to express a substance, such as a receptor, which binds to the protein of the present invention (for example, vascular endothelial cells, bone marrow cells, or lymph duct cells), introducing it into such cells as COS, detecting the binding of the protein of the present invention by itself or labeled with a radioisotope or a fluorescence, and cloning proteins that bind to the protein of the present invention (Yamasaki, K., Taga, T., Hirata, Y., Yawata, H., Kawanishi, Y., Seed, B., Taniguchi, T., Hirano, T., and Kishimoto, T. (1988) Cloning and expression of human interleukin-6 (BSF-2/IFN beta2) receptor, *Science* 241: 825-828, Fukunaga, R., Ishizaka-Ikeda, E., Seto, Y., and Nagata, S. (1990) Expression cloning of a receptor for murine granulocyte colony-stimulating factor, *Cell* 61: 341-350). Still another method comprises applying the culture supernatant or the cellular extract of the cells expected to express a protein that binds to the protein of the present invention onto an affinity column to which the protein of the present invention has been immobilized, and purifying the proteins specifically bound to the column. In addition, a DNA encoding the protein that binds to the protein of the present invention can be obtained by determining the amino acid sequence of the binding protein, synthesizing oligonucleotides based on the sequence, and screening a cDNA library with the oligonucleotides as probes.

[0021] Furthermore, compounds that bind to the protein of the present invention can be screened by contacting compounds, a natural substance bank, or a random phage peptide display library with the immobilized protein of the present invention and detecting the molecules bound to the protein. These compounds can also be screened by high throughput screening utilizing combinatorial chemistry technology (Wrighton, N. C., Farrell, F. X., Chang, R., Kashyap, A. K., Barbone, F. P., Mulcahy, L. S., Johnson, D. L., Barrett, R. W., Jolliffe, L. K., and Dower, W. J., Small peptides as potent mimetics of the protein hormone erythropoietin, *Science (United States)* Jul 26 1996, 273: 458-464, Verdine, G.L., The combinatorial chemistry of nature, *Nature (England)* Nov 7 1996, 384: 11-13, Hogan, J.C. Jr. Directed combinatorial chemistry, *Nature (England)* Nov 7 1996, 384: 17-19).

[0022] VEGF-D of the present invention may be used for gene therapy by introducing the VEGF-D gene into the body of the patient with the VEGF-D deficiency, or expressing the gene in the body. An anti-sense DNA of the VEGF-D gene may also be used to inhibit the expression of the gene itself, thereby suppressing the pathological neovascularization.

[0023] Among the many available methods to introduce the VEGF-D gene or its antisense DNA into the body, the retrovirus method, the liposome method, the cationic liposome method, and the adenovirus method are preferable.

[0024] In order to express these genes in the body, the genes can be incorporated into a suitable vector and introduced into the body by the retrovirus method, the liposome method, the cationic liposome method, or the adenovirus method. Although the vectors to be used are not particularly limited, such vectors as pAdexlcw and pZIPneo are preferable.

5 [0025] The present invention may also be applied for diagnosing disorders caused by abnormalities of the VEGF-D gene, for example, by PCR to detect an abnormality of the nucleotide sequence of the VEGF-D gene.

[0026] Furthermore, according to the present invention, the VEGF-D protein or its agonists can be used to heal wounds, promote collateral vessel formation, and aid hematopoiesis by the hematopoietic stem cells, by taking advantage of the angiogenic effect of the VEGF-D protein. The antibodies against the VEGF-D protein or its antagonists can 10 be used as the therapeutic agents for pathological neovascularization, lymphatic dysplasia, dyshematopoiesis, or edemas arising from various causes. The anti-VEGF-D antibodies can be used for diagnosing diseases resulting from abnormal production of VEGF-D by quantifying VEGF-D.

Brief Description of the Drawings

15

[0027]

Figure 1 shows the relationship among the VEGF-D gene, the EST sequences, and the primers used for cloning. Figure 2 compares the amino acid sequences of EST (H24828) and VEGF-C.

20 Figure 3 compares the amino acid sequences deduced from the VEGF-D gene and from the known genes of the VEGF family proteins.

Figure 4a shows the hydrophobicity plot of VEGF-D. Figure 4b shows the prediction of the cleavage site of the VEGF-D signal peptide.

25 [0028] Best Mode for Implementing the Invention

[0028] The following examples illustrate the present invention in detail, but are not to be construed to limit the scope of the invention.

30 Example 1. Homology search by TFASTA method

[0029] The sequence CGPNKELDENTCQCVC (SEQ ID NO. 3) was designed based on the consensus sequence found in the BR3P (Balbiani ring 3 protein) repeat at the C-terminus of VEGF-C. The entire ESTs and STS sequences in the Genbank database (as of 29 February 1996) were then searched by the TFASTA method (Person and Lipman, 35 Proc. Natl. Acad. Sci. USA 85: 2444-2448 (1988)). The searching conditions used are shown below (Table 1).

Table 1

Sequences	392,210
Symbols	135,585,305
Word Size	2
Gap creation penalty	12.0
Gap extension penalty	4.0

40

[0030] As a result, an EST (Accession No. H24828) that is considered to code the consensus sequence was found. The sequence is one of the ESTs registered by The WashU-Merck EST Project, and nine out of 16 amino acid residues 50 were identical. Further searching for UniGene by NCBI based on this sequence revealed that five registered sequences (T64149, H24780, H24633, H24828, and T64277 (as of 1 March 1996)), including the above EST, were considered to be derived from the same gene. T64277 and T64149, as well as H24828 and H24780, are the combination of the 5' sequence and the 3' sequence of the same clones, and the length of the insert in both of these clones was 0.9 kb (Fig. 1).

55

[0031] Translating the H24828 sequence into a protein sequence in a frame where homology is found suggested that this sequence codes 104 C-terminal amino acid residues. Comparing this amino acid sequence with the C-terminus of VEGF-C, 28 out of 104 amino acids (27%) were identical. Moreover, the amino acids that are important for maintaining the protein structure, such as cysteine and proline, were well conserved (Fig. 2). Conserved sequences are shown in a

black box.

Example 2. cDNA cloning from a library

5 [0032] Primers for 5' RACE and 3' RACE (5' RACE primer: 5'-AGGGATGGGAACTTGGAACGCTGAAT-3' (SEQ ID NO. 4), 3' RACE primer: 5'-GATCTAATCCAGCACCCAAAACGTGC-3'(SEQ ID NO. 5)) were designed (Fig. 1). A double-stranded cDNA was synthesized from human lung-derived polyA⁺ RNA using reverse transcriptase. PCR was then performed using Marathon-Ready cDNA, Lung (Chlontech), having an adapter cDNA ligated to both ends as a template cDNA, and using the above primer and adapter primer (AP-1 primer) as primers. The above adapter cDNA contains the
 10 regions to which the adapter primers AP-1 and AP-2 hybridize. The PCR was performed in a manner such that the system was exposed to treatment at 94°C for 1 min; five cycles of treatment at 94°C for 30 sec and at 72°C for 4 min; five cycles of treatment at 94°C for 30 sec and at 70°C for 4 min; then 25 cycles of treatment at 94°C for 20 sec and at 68°C for 4 min. (TaKaRa Ex Taq (Takara Shuzo) and the attached buffer were used as Taq polymerase instead of Advantage KlenTaq Polymerase Mix.) As a result, 1.5kb fragments were amplified at the 5' region and 0.9kb fragments at the 3'
 15 region. These fragments were cloned with the pCR-Direct Cloning System (Clontech), CR-TRAP Cloning System (Gen-Hunter), and PT7Blue-T vector (Novagen). When the 5'-RACE fragment was cloned into the pCR-Direct vector, the fragment was amplified again using 5'-CTGGTCGGCCCAGAACGCTGAATCA-3' (SEQ No. 7) and 5'-
 20 CTCGCTGCCACTAATACGACTCACTATAGG-3' (SEQ ID NO. 8) as primers.

20 **Example 3. Nucleotide sequence analysis**

25 [0033] ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase FS and 377 A DNA Sequencer (ABI) were used for DNA sequencing. The primers used are the primers in the vectors (5'-AATTAAC-
 20 CCTCACTAAAGGG-3' (SEQ ID NO. 9), 5'-CCAGGGTTTCCAGTCACGAC-3'(SEQ ID NO. 10)), AP-2 primer (5'-
 25 ACTCACTATAAGGCTCGAGCGGC-3' (SEQ ID NO. 11)), and 10 primers in the sequence shown below (Table 2).

Table 2

SQ1 (SEQ ID NO. 12)	5'-AAGTCTGGAGACCTGCT-3'
SQ2 (SEQ ID NO. 13)	5'-CAGCAGGTCTCCAGACT-3'
SQ3 (SEQ ID NO. 14)	5'-CGCACCCAAGGAATGGA-3'
SQ4 (SEQ ID NO. 15)	5'-TGACACCTGGCCATTCCA-3'
SQ5 (SEQ ID NO. 16)	5'-CATCAGATGGTAGTTCAT-3'
SQ6 (SEQ ID NO. 17)	5'-ATGCTGAGCGAGAGTCCATA-3'
SQ7 (SEQ ID NO. 18)	5'-CACTAGGTTGGCAAGCACTTACA-3'
SQ8 (SEQ ID NO. 19)	5'-GCTGTTGGCAAGCACTTACA-3'
SQ9 (SEQ ID NO. 20)	5'-GATCCATCCAGATCCCTGAA-3'
SQ10 (SEQ ID NO. 21)	5'-CAGATCAGGGCTGCTTCTA-3'

45 [0034] Determining the nucleotide sequence of the 1.5kb fragment at the 5'-side and the 0.9kb fragment at the 3'-side revealed that the sequence of the overlapping region was identical, confirming that 5'- and 3'-side cDNAs of the desired gene were obtained. Determining the entire nucleotide sequence of the cDNA revealed that this novel gene has the full length of 2 kb and can code a protein consisting of 354 amino acid residues (SEQ ID NO. 1 and SEQ ID NO. 2). Figure 1 shows the relation between this gene and the EST sequences registered in the Genbank database. Comparing the
 50 amino acid sequence with other VEGF family proteins revealed that the amino acids that are well conserved between family proteins are also conserved in this novel gene, and therefore this gene is obviously a new member of the VEGF family (Fig. 3). In Fig. 3, HSVEGF indicates human VEGF; HSVEGF-D, HSVEGF-C, and HSVEGF-B indicate human VEGF homologues (human VEGF-D, human VEGF-C, and human YEGF-B, respectively); HSPDGFA indicates human PDGF-A; HSPDGFB indicates human PDGF-B; and HSP1GF2 indicates human P1GF2. The conserved sequences are shown in a black box. Since VEGF-D is highly homologous to VEGF-C that was cloned as the Flt4 ligand, it was presumed to be a ligand to a Flt-4-like receptor.
 55 [0035] Deducing the signal peptide cleavage site (Fig. 4b) by hydrophobicity plot (Fig. 4a) and the method of von Heijne (von Heijne, G, Nucleic Acids Res. 14, 4683-4690(1986)), N-terminal 21 amino acid residues may be cleaved as

signal peptides, and they may also undergo additional processing like VEGF-C.

Example 4. Northern blot analysis

5 [0036] A 1kb fragment, which had been cut out by digestion with EcoRI from the 5'-fragment subcloned into pCR-Direct vector, was labeled with [α -³²P]dCTP and used as a probe. Labeling was performed by random priming using Ready-to Go DNA labeling beads (Pharmacia). Hybridization was performed in ExpressHyb Hybridization Solution (Clontech) by the usual method using Multiple Tissue Northern (MTN) Blot-Human, Human II, Human Fetal, and Human Cell lines (Clontech). Significant expression was observed in lung, heart, and intestine. Weak expression was
10 observed in skeletal muscle, ovary, colon, and pancreas. The apparent molecular weight of the mRNA was 2.2 kb, and the cloned fragment seemed to be almost the full length of the gene.

Example 5. VEGF-D protein expression in E. coli

15 [0037] Two primers, 5'-TCCAGATCTTTGCGGCAACTTCTATGACAT-3' (SEQ ID NO. 22) and 5'-CAGGTCGACT-CAAACAGGCACATTCAAGGTAC-3' (SEQ ID NO. 23), were synthesized to amplify the region corresponding to the 89th to 181st amino acid residues of human VEGF cDNA. The thus-obtained DNA fragment was digested with restriction enzymes BglII and Sall, and ligated using ligation kit II (Takara Shuzo Co., Ltd) to plasmid pQE42 ((QIAGEN), which had been digested with restriction enzymes BamHI and Sall. The resulting plasmid was introduced into E. coli
20 SG19003[pREP4] (QIAGEN), and a plasmid, which was obtained as designed without any mutation, was selected (pQE42-BS3). Plasmid pQE42-BS3 was introduced into E. coli BL21 (Invitrogen) and cultured in 10 ml of L Broth containing 100 mg/l bicucilline (ampicillin sodium for injection, Meiji Seika Kaisha, Ltd.). 200 ml of fresh L Broth was then inoculated with the culture. After incubation at 37°C for 1.5 hours, IPTG was added to 3 mM, and the culture was further incubated at 37°C for 5 hours. After cells were harvested, a protein was purified with a Ni-NTA column following the protocol of QIAexpress Typell kit.
25

Example 6. Expression of DHFR-VEGF-D fusion protein in E. coli

30 [0038] The region corresponding to the 89th to 181st amino acid residues of human VEGF cDNA was amplified with the same primers used in Example 5. The thus-obtained DNA fragment was digested with restriction enzymes BglII and Sall. The fragment was then ligated using ligation kit II (Takara Shuzo Co., Ltd.) to the plasmid pQE40 (QIAGEN), which had been digested with restriction enzymes BamHI and Sall. The resulting plasmid was introduced into E. coli SG19003[pREP4] (QIAGEN), and a plasmid, which was obtained as designed without any mutation, was selected (pQE40-BS3). Plasmid pQE40-BS3 was introduced into E. coli BL21 (Invitrogen) and cultured in 10 ml of L Broth containing 100 mg/l bicucilline (ampicillin sodium for injection, Meiji Seika Kaisha, Ltd.). 200 ml of fresh L Broth was then
35 inoculated with the culture. After incubation at 37°C for 1.5 hours, IPTG was added to 3 mM, and the culture was further incubated at 37°C for 5 hours. After cells were harvested, a DHFR-VEGF-D fusion protein was purified with a Ni-NTA column following the protocol of a QIAexpress Typell kit.

40 **Example 7. Cloning mouse VEGF-D cDNA**

45 [0039] Two Hybond-N+ (Amersham) filters (20 cm x 22 cm) on which 1.5×10^5 pfu of Mouse lung 5'-stretch cDNA library was transferred were prepared. Gradient hybridization from 68°C to 55°C was performed for 2 hours in ExpressHyb Hybridization Solution (Clontech) using as a probe an approximately 50 ng Pvu II fragment of human VEGF-D, which had been labeled with [α -³²P]-dCTP (Amersham) using Ready-To-Go DNA Labeling Beads(-dCTP) (Pharmacia). The filters were washed four times in 2 x SSC, 0.05% SDS at room temperature for 10 min, then washed in 0.1 x SSC, 0.1% SDS at 45°C for 3 min. The washed filters were exposed overnight at -80°C using HyperFilm MP (Amersham) and intensifying paper. Positive clones were subjected to the second screening in the same manner as above to isolate a single clone. Isolated lambda DNAs were purified from the plate lysate using a QIAGEN Lambda MAX I Kit (Qiagen). Insert DNAs were cut out with EcoRI and subcloned into pUC118 EcoRI/BAP (Takara Shuzo Co., Ltd.). Its nucleotide sequence was then determined with ABI377 sequencer (Perkin Elmer). The cDNA coding the full length of mouse VRGF-D was reconstructed with two of the obtained clones that overlapped each other. SEQ ID NO. 24 shows the nucleotide sequence of mouse VEGF-D cDNA and the deduced amino acid sequence therefrom.

55 **Example 8. Cloning rat VEGF-D cDNA**

[0040] Two Hybond-N+ (Amersham) filters (20 cm x 22 cm), on which 1.5×10^5 pfu of Rat lung 5'-stretch cDNA library had been transferred, were prepared. Gradient hybridization from 68°C to 55°C was performed for 2 hours in

EP 0 935 001 A1

ExpressH.Fyb Hybridization Solution (Clontech) using as a probe an approximately 1 µg fragment containing 1-782 bp of the mouse VEGF-D cDNA which had been labeled with $\alpha^{32}\text{P}$ -dCTP (Amersham) using Ready-To-Go DNA Labeling Beads(-dCTP) (Pharmacia). The filters were washed four times in 2 x SSC, 0.05% SDS at room temperature for 10 min, then washed in 0.1 x SSC, 0.1% SDS at 45°C for 3 min. The washed filters were exposed overnight at -80°C using HyperFilm MP (Amersham) and intensifying paper. Positive clones were subjected to the second screening in the same manner as above to isolate a single clone. The isolated positive clone was excised into pBluescript using E. coli SOLAR (Stratagene) and helper phage ExAssist (Stratagene), then the sequence was determined with ABI377 sequencer (Perkin Elmer). The sequence seemed to be the rat VEGF-D cDNA but did not contain the termination codon.

5 [0041] To obtain the C-terminal cDNA which had not been obtained, PCR was performed using Marathon-Ready rat kidney cDNA (Clontech) as a template and 5' primer GCTGCGAGTGTGCTGTAAA (SEQ ID NO. 26) and 3' primer GGGTAGTGGCACACAGTGACAGCAA (SEQ ID NO. 27) with 40 cycles of 94°C for 15 sec, 55°C for 30 sec, and 72 °C for 2 min. After the thus-obtained fragment was subcloned into pGEM-T vector (promega), the nucleotide sequence was determined with ABI377 sequencer (Perkin Elmer). The resulting clone contained the C-terminus of rat VEGF-D. Based on the results of sequencing the clone obtained by plaque hybridization and the clone obtained by PCR, the full 15 length of the rat VEGF-D sequence was determined. SEQ ID NO. 25 shows the determined nucleotide sequence and the deduced amino acid sequence therefrom.

Industrial Applicability

20 [0042] In the present invention, a novel protein (VEGF-D) having significant homology to VEGF-C and its gene have been isolated. VEGF-D appears to be involved in the pathological neovascularization associated with diabetes, rheumatoid arthritis, the growth of solid tumors, differentiation and proliferation of blood cells, formation of lymphatic vessels, and formation of edema resulting from various causes as well as the normal neovascularization at the developmental stage. The gene of the present invention can be used to diagnose disorders caused by abnormalities of the VEGF-D 25 gene and gene therapy for the VEGF-D deficiency. The VEGF-D protein, which is obtained by expressing the gene of the present invention, can be used for healing wounds, promoting collateral vessel formation, and aiding hematopoietic stemcell proliferation. The antibodies or inhibitors against the VEGF-D protein can be used for treating angiodyplasia and lymphangiodysplasia associated with inflammation, edemas arising from various causes, dyshematopoiesis, and, as a novel anticancer agent, for treating pathological neovascularization. The VEGF-D protein and its antibodies can be 30 useful for diagnosing diseases resulting from abnormal production of VEGF-D.

35

40

45

50

55

Sequence Listing

5 (1) Name or appellation of Applicant: Chugai Research Institute for
Molecular Medicine, Inc.

(2) Title of the Invention: Novel VEGF-like Factor

(3) Reference Number: C1-802PCT

10 (4) Application Number:

(5) Filing date:

(6) Country where the priority application was filed and the
15 application number of the application: Japan, No. Hei 8-185216

(7) Priority date: July 15, 1996

(8) Number of sequences: 27

20 SEQ ID NO: 1

SEQUENCE LENGTH: 354

SEQUENCE TYPE: amino acid

25 TOPOLOGY: linear

MOLECULE TYPE: protein

ORIGINAL SOURCE:

ORGANISM: Homo sapiens

30 TISSUE TYPE: lung

SEQUENCE DESCRIPTION:

Met Tyr Arg Glu Trp Val Val Val Asn Val Phe Met Met Leu Tyr Val

35 1 5 10 15

Gln Leu Val Gln Gly Ser Ser Asn Glu His Gly Pro Val Lys Arg Ser

20 25 30

Ser Gln Ser Thr Leu Glu Arg Ser Glu Gln Ile Arg Ala Ala Ser

40 35 40 45

Ser Leu Glu Glu Leu Leu Arg Ile Thr His Ser Glu Asp Trp Lys Leu

50 55 60

Trp Arg Cys Arg Leu Arg Leu Lys Ser Phe Thr Ser Met Asp Ser Arg

45 65 70 75 80

Ser Ala Ser His Arg Ser Thr Arg Phe Ala Ala Thr Phe Tyr Asp Ile

85 90 95

50 Glu Thr Leu Lys Val Ile Asp Glu Glu Trp Gln Arg Thr Gln Cys Ser

100 105 110

55

EP0935001 A1

Pro Arg Glu Thr Cys Val Glu Val Ala Ser Glu Leu Gly Lys Ser Thr
115 120 125
5 Asn Thr Phe Phe Lys Pro Pro Cys Val Asn Val Phe Arg Cys Gly Gly
130 135 140
Cys Cys Asn Glu Glu Ser Leu Ile Cys Met Asn Thr Ser Thr Ser Tyr
145 150 155 160
10 Ile Ser Lys Gln Leu Phe Glu Ile Ser Val Pro Leu Thr Ser Val Pro
165 170 175
Glu Leu Val Pro Val Lys Val Ala Asn His Thr Gly Cys Lys Cys Leu
180 185 190
15 Pro Thr Ala Pro Arg His Pro Tyr Ser Ile Ile Arg Arg Ser Ile Gln
195 200 205
Ile Pro Glu Glu Asp Arg Cys Ser His Ser Lys Lys Leu Cys Pro Ile
210 215 220
20 Asp Met Leu Trp Asp Ser Asn Lys Cys Lys Cys Val Leu Gln Glu Glu
225 230 235 240
Asn Pro Leu Ala Gly Thr Glu Asp His Ser His Leu Gln Glu Pro Ala
245 250 255
25 Leu Cys Gly Pro His Met Met Phe Asp Glu Asp Arg Cys Glu Cys Val
260 265 270
30 Cys Lys Thr Pro Cys Pro Lys Asp Leu Ile Gln His Pro Lys Asn Cys
275 280 285
Ser Cys Phe Glu Cys Lys Glu Ser Leu Glu Thr Cys Cys Gln Lys His
290 295 300
35 Lys Leu Phe His Pro Asp Thr Cys Ser Cys Glu Asp Arg Cys Pro Phe
305 310 315 320
His Thr Arg Pro Cys Ala Ser Gly Lys Thr Ala Cys Ala Lys His Cys
325 330 335
40 Arg Phe Pro Lys Glu Lys Arg Ala Ala Gln Gly Pro His Ser Arg Lys
340 345 350
45 Asn Pro

SEQ ID NO: 2

SEQUENCE LENGTH: 2004

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

MOLECULE TYPE: cDNA to mRNA

5 ORIGINAL SOURCE:

ORGANISM: Homo sapiens

TISSUE TYPE: lung

FEATURE:

10 NAME/KEY: CDS

LOCATION: 403..1464

IDENTIFICATION METHOD: E

15 SEQUENCE DESCRIPTION:

CCAGCTTTCT	GTARCTGAA	GCATTGGTGG	CCACACCACC	TCCTTACAAA	GCAACTAGAA	60
CCTGCGGCAT	ACATTGGAGA	GATTTTTTA	ATTTCTGGA	CAYGAAGTAA	ATTTAGAGTG	120
CTTTCYAATT	TCAGGTACAA	GACATGTCCA	CCTTCTGATT	ATTTTGGAG	AACATTTGA	180
20 TTTTTTCAT	CTCTCTCTCC	CCACCCCTAA	GATTGTCAA	AAAAAGCGTA	CCTTGCTAA	240
TTGAAATAAT	TTCATTGGAT	TTTGATCAGA	ACTGATCATT	TGGTTTCTG	TGTGAAGTTT	300
TGAGGTTCA	AACTTCCCTT	CTGGAGAATG	CCTTTGAAA	CAATTTCTC	TAGCTGCCG	360
25 ATGTCAACTG	CTTAGTAATC	AGTGGATATT	AAAATATTCA	AA ATG TAC AGA GAG		414

Met Tyr Arg Glu

1

30 TGG	GTA	GTG	GTG	AAT	GTT	TTC	ATG	ATG	TTG	TAC	GTC	CAG	CTG	GTG	CAG	462
Trp	Val	Val	Val	Asn	Val	Phe	Met	Met	Leu	Tyr	Val	Gln	Leu	Val	Gln	
5																
35 GGC	TCC	AGT	AAT	GAA	CAT	GGA	CCA	GTG	AAG	CGA	TCA	TCT	CAG	TCC	ACA	510
Gly	Ser	Ser	Asn	Glu	His	Gly	Pro	Val	Lys	Arg	Ser	Ser	Gln	Ser	Thr	
40 TTG	GAA	CGA	TCT	GAA	CAG	CAG	ATC	AGG	GCT	GCT	TCT	AGT	TTG	GAG	GAA	558
Leu	Glu	Arg	Ser	Glu	Gln	Gln	Ile	Arg	Ala	Ala	Ser	Ser	Leu	Glu	Glu	
45 CTA	CTT	CGA	ATT	ACT	CAC	TCT	GAG	GAC	TGG	AAG	CTG	TGG	AGA	TGC	AGG	606
Leu	Leu	Arg	Ile	Thr	His	Ser	Glu	Asp	Trp	Lys	Leu	Trp	Arg	Cys	Arg	
50 CTG	AGG	CTC	AAA	AGT	TTT	ACC	AGT	ATG	GAC	TCT	CGC	TCA	GCA	TCC	CAT	654
Leu	Arg	Leu	Lys	Ser	Phe	Thr	Ser	Met	Asp	Ser	Arg	Ser	Ala	Ser	His	
55 CGG	TCC	ACT	AGG	TTT	CCG	GCA	ACT	TTC	TAT	GAC	ATT	GAA	ACA	CTA	AAA	702
Arg	Ser	Thr	Arg	Phe	Ala	Ala	Thr	Phe	Tyr	Asp	Ile	Glu	Thr	Leu	Lys	

EP 0 935 001 A1

	85	90	95	100	
	GTT ATA GAT GAA GAA TGG CAA AGA ACT CAG TGC AGC CCT AGA GAA ACG				750
5	Val Ile Asp Glu Glu Trp Gln Arg Thr Gln Cys Ser Pro Arg Glu Thr				
	105	110	115		
	TGC GTG GAG GTG GCC AGT GAG CTG GGG AAG AGT ACC AAC ACA TTC TTC				798
	Cys Val Glu Val Ala Ser Glu Leu Gly Lys Ser Thr Asn Thr Phe Phe				
10	120	125	130		
	AAG CCC CCT TGT GTG AAC GTG TTC CGA TGT GGT GGC TGT TGC AAT GAA				846
	Lys Pro Pro Cys Val Asn Val Phe Arg Cys Gly Cys Cys Asn Glu				
15	135	140	145		
	GAG AGC CTT ATC TGT ATG AAC ACC AGC ACC TCG TAC ATT TCC AAA CAG				894
	Glu Ser Leu Ile Cys Met Asn Thr Ser Thr Ser Tyr Ile Ser Lys Gln				
20	150	155	160		
	CTC TTT GAG ATA TCA GTG CCT TTG ACA TCA GTA CCT GAA TTA GTG CCT				942
	Leu Phe Glu Ile Ser Val Pro Leu Thr Ser Val Pro Glu Leu Val Pro				
	165	170	175	180	
25	GTT AAA GTT GCC AAT CAT ACA GGT TGT AAG TGC TTG CCA ACA GCC CCC				990
	Val Lys Val Ala Asn His Thr Gly Cys Lys Cys Leu Pro Thr Ala Pro				
	185	190	195		
30	CGC CAT CCA TAC TCA ATT ATC AGA AGA TCC ATC CAG ATC CCT GAA GAA				1038
	Arg His Pro Tyr Ser Ile Ile Arg Arg Ser Ile Gln Ile Pro Glu Glu				
	200	205	210		
	GAT CGC TGT TCC CAT TCC AAG AAA CTC TGT CCT ATT GAC ATG CTA TGG				1086
35	Asp Arg Cys Ser His Ser Lys Lys Leu Cys Pro Ile Asp Met Leu Trp				
	215	220	225		
	GAT AGC AAC AAA TGT AAA TGT GTT TTG CAG GAG GAA AAT CCA CTT GCT				1134
	Asp Ser Asn Lys Cys Lys Cys Val Leu Gln Glu Glu Asn Pro Leu Ala				
40	230	235	240		
	GGA ACA GAA GAC CAC TCT CAT CTC CAG GAA CCA GCT CTC TGT GGG CCA				1182
	Gly Thr Glu Asp His Ser His Leu Gln Glu Pro Ala Leu Cys Gly Pro				
45	245	250	255	260	
	CAC ATG ATG TTT GAC GAA GAT CGT TGC GAG TGT GTC TGT AAA ACA CCA				1230
	His Met Met Phe Asp Glu Asp Arg Cys Glu Cys Val Cys Lys Thr Pro				
	265	270	275		
50	TGT CCC AAA GAT CTA ATC CAG CAC CCC AAA AAC TGC AGT TGC TTT GAG				1278
	Cys Pro Lys Asp Leu Ile Gln His Pro Lys Asn Cys Ser Cys Phe Glu				

EP 0 935 001 A1

	280	285	290	
	TGC AAA GAA AGT CTG GAG ACC TGC TGC CAG AAG CAC AAG CTA TTT CAC			1326
5	Cys Lys Glu Ser Leu Glu Thr Cys Cys Gln Lys His Lys Leu Phe His			
	295	300	305	
	CCA GAC ACC TGC AGC TGT GAG GAC AGA TGC CCC TTT CAT ACC AGA CCA			1374
10	Pro Asp Thr Cys Ser Cys Glu Asp Arg Cys Pro Phe His Thr Arg Pro			
	310	315	320	
	TGT GCA AGT GGC AAA ACA GCA TGT GCA AAG CAT TGC CGC TTT CCA AAG			1422
	Cys Ala Ser Gly Lys Thr Ala Cys Ala Lys His Cys Arg Phe Pro Lys			
15	325	330	335	340
	GAG AAA AGG GCT GCC CAG GGG CCC CAC AGC CGA AAG AAT CCT			1464
	Glu Lys Arg Ala Ala Gln Gly Pro His Ser Arg Lys Asn Pro			
20	345	350		
	TGATTCAAGCG TTCCAAGTTC CCCATCCCTG TCATTTTAA CAGCATGCTG CTTTCCAAG			1524
	TTGCTGTCAC TGTTTTTTC CCAGGTGTTA AAAAAAAAAT CCATTTACA CAGCACCAACA			1584
25	GTGAATCCAG ACCAACCTTC CATTACACCC AGCTAAGGAG TCCCTGGTTC ATTGATGGAT			1644
	GTCTCTAGC TGCAAGATGCC TCTGCCACC AAGGAATGGA GAGGAGGGGA CCCATGTAAT			1704
	CCTTTGTTT AGTTTGTTT TTGTTTTTG GTGAATGAGA AAGGTGTGCT GGTATGGAA			1764
	TGGCAGGTGT CATATGACTG ATTACTCAGA GCAGATGAGG AAAACTGTAG TCTCTGAGTC			1824
30	CTTGCTAAT CGCAACTCTT GTGAATTATT CTGATTCTTT TTTATGCAGA ATTTGATTG			1884
	TATGATCAGT ACTGACTTTC TGATTACTGT CCAGCTTATA GTCTCCAGT TTAATGAAC			1944
	ACCATCTGAT GTTTCATATT TAAGTGTATT TAAAGAAAAT AAACACCATT ATTCAAGTCT			2004

35 SEQ ID NO: 3

SEQUENCE LENGTH: 16

SEQUENCE TYPE: amino acid

40 TOPOLOGY: linear

MOLECULE TYPE: peptide

ORIGINAL SOURCE:

45 ORGANISM: Homo sapiens

TISSUE TYPE: lung

SEQUENCE DESCRIPTION:

Cys Gly Pro Asn Lys Glu Leu Asp Glu Asn Thr Cys Gln Cys Val Cys

50 1 5 10 15

55

SEQ ID NO: 4
SEQUENCE LENGTH: 27
5 SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
10 MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
AGGGATGGGG AACTTGGAAC GCTGAAT 27

15 SEQ ID NO: 5
SEQUENCE LENGTH: 27
SEQUENCE TYPE: nucleic acid
20 STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
25 GATCTAATCC AGCACCCCCAA AAACTGC 27

30 SEQ ID NO: 6
SEQUENCE LENGTH: 27
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
35 TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
CCATCCTAAT ACGACTCACT ATAGGGC 27

40 SEQ ID NO: 7
SEQUENCE LENGTH: 33
SEQUENCE TYPE: nucleic acid
45 STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
50 SEQUENCE DESCRIPTION:
CTGGTTCGGC CCAGAACTTG GAACGCTGAA TCA 33

SEQ ID NO: 8
SEQUENCE LENGTH: 32
5 SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
10 MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
CTCGCTGCC CACTAATACG ACTCACTATA GG 32

15 SEQ ID NO: 9
SEQUENCE LENGTH: 20
SEQUENCE TYPE: nucleic acid
20 STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
25 AATTAACCCT CACTAAAGGG 20

30 SEQ ID NO: 10
SEQUENCE LENGTH: 22
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
35 TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
CCAGGGTTTT CCCAGTCACG AC 22

40 SEQ ID NO: 11
SEQUENCE LENGTH: 23
SEQUENCE TYPE: nucleic acid
45 STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
50 SEQUENCE DESCRIPTION:
ACTCACTATA GGGCTCGAGC GGC 23

EP 0 935 001 A1

SEQ ID NO: 12
SEQUENCE LENGTH: 17
5 SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
10 MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
AAGTCTGGAG ACCTGCT 17

15 SEQ ID NO: 13
SEQUENCE LENGTH: 17
SEQUENCE TYPE: nucleic acid
20 STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
25 CAGCAGGTCT CCAGACT 17

SEQ ID NO: 14
30 SEQUENCE LENGTH: 17
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
35 TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
CGCACCCAAG GAATGGA 17

40 SEQ ID NO: 15
SEQUENCE LENGTH: 18
45 SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
50 SEQUENCE DESCRIPTION:
TGACACCTGG CCATTCCA 18

55
SEQ ID NO: 16
SEQUENCE LENGTH: 18
5 SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
10 MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
CATCAGATGG TAGTTCAT 18

15 SEQ ID NO: 17
SEQUENCE LENGTH: 20
SEQUENCE TYPE: nucleic acid
20 STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
25 ATGCTGAGCG AGAGTCCATA 20

30 SEQ ID NO: 18
SEQUENCE LENGTH: 20
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
35 TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
CACTAGGTTT GCGGCAACTT 20
40
45 SEQ ID NO: 19
SEQUENCE LENGTH: 20
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
50 MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
GCTGTTGGCA AGCACTTACA 20
55

EP 0 935 001 A1

SEQ ID NO: 20
SEQUENCE LENGTH: 20
5 SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
10 MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
GATCCATCCA GATCCCTGAA 20

15 SEQ ID NO: 21
SEQUENCE LENGTH: 19
SEQUENCE TYPE: nucleic acid
20 STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
25 SEQUENCE DESCRIPTION:
CAGATCAGGG CTGCTTCTA 19

30 SEQ ID NO: 22
SEQUENCE LENGTH: 32
SEQUENCE TYPE: nucleic acid
35 STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE DESCRIPTION:
TCCAGATCTT TTGCGGCAAC TTTCTATGAC AT 32

40 SEQ ID NO: 23
SEQUENCE LENGTH: 33
45 SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
50 SEQUENCE DESCRIPTION:
CAGGTGCACT CAAACAGGCA CTAATTCAAGG TAC 33

55

EP 0 935 001 A1

SEQ ID NO: 24
SEQUENCE LENGTH: 1581
5 SEQUENCE TYPE: nucleic acid
STRANDEDNESS: double
TOPOLOGY: linear
10 MOLECULE TYPE: cDNA to mRNA
ORIGINAL SOURCE:
ORGANISM: mouse
TISSUE TYPE: lung
15 FEATURE:
NAME/KEY: CDS
LOCATION: 96..1169
IDENTIFICATION METHOD: E
20 SEQUENCE DESCRIPTION:
TTCCGGGCTT TGCTGGAGAA TGCCTTTGC AACACTTTTC AGTAGCTGCC TGGAAACAAC 60
TGCTTAGTCA TCGGTAGACA TTTAAAATAT TCAAA ATG TAT GGA GAA TGG GGA 113
Met Tyr Gly Glu Trp Gly
25 1 5
ATG GGG AAT ATC CTC ATG ATG TTC CAT GTG TAC TTG GTG CAG GGC TTC 161
Met Gly Asn Ile Leu Met Met Phe His Val Tyr Leu Val Gln Gly Phe
30 10 15 20
AGG AGC GAA CAT GGA CCA GTG AAG GAT TTT TCT TTT GAG CGA TCA TCC 209
Arg Ser Glu His Gly Pro Val Lys Asp Phe Ser Phe Glu Arg Ser Ser
35 25 30 35
CGG TCC ATG TTG GAA CGA TCT GAA CAA CAG ATC CGA GCA GCT TCT AGT 257
Arg Ser Met Leu Glu Arg Ser Glu Gln Gln Ile Arg Ala Ala Ser Ser
40 40 45 50
45 TTG GAG GAG TTG CTG CAA ATC GCG CAC TCT GAG GAC TGG AAG CTG TGG 305
Leu Glu Glu Leu Leu Gln Ile Ala His Ser Glu Asp Trp Lys Leu Trp
55 55 60 65 70
CGA TGC CGG TTG AAG CTC AAA AGT CTT GCC AGT ATG GAC TCA CGC TCA 353
Arg Cys Arg Leu Lys Leu Lys Ser Leu Ala Ser Met Asp Ser Arg Ser
75 75 80 85
50 GCA TCC CAT CGC TCC ACC AGA TTT GCG GCA ACT TTC TAT GAC ACT GAA 401
Ala Ser His Arg Ser Thr Arg Phe Ala Ala Thr Phe Tyr Asp Thr Glu
90 90 95 100

EP 0 935 001 A1

	ACA CTA AAA GTT ATA GAT GAA GAA TGG CAG AGG ACC CAA TGC AGC CCT	449
	Thr Leu Lys Val Ile Asp Glu Glu Trp Gln Arg Thr Gln Cys Ser Pro	
5	105 110 115	
	AGA GAG ACA TGC GTC GAA GTC GCC AGT GAG CTG GGG AAG ACA ACC AAC	497
	Arg Glu Thr Cys Val Glu Val Ala Ser Glu Leu Gly Lys Thr Thr Asn	
10	120 125 130	
	ACA TTC TTC AAG CCC CCC TGT GTC AAT GTC TTC CGG TGT GGA GGC TGC	545
	Thr Phe Phe Lys Pro Pro Cys Val Asn Val Phe Arg Cys Gly Gly Cys	
15	135 140 145 150	
	TGC AAC GAA GAG GGT GTG ATG TGT ATG AAC ACA AGC ACC TCC TAC ATC	593
	Cys Asn Glu Gly Val Met Cys Met Asn Thr Ser Thr Ser Tyr Ile	
	155 160 165	
20	TCC AAA CAG CTC TTT GAG ATA TCA GTG CCT CTG ACA TCA GTG CCC GAG	641
	Ser Lys Gln Leu Phe Glu Ile Ser Val Pro Leu Thr Ser Val Pro Glu	
	170 175 180	
	TTA GTG CCT GTT AAA ATT GCC AAC CAT ACG GGT TGT AAG TGC TTG CCC	689
25	Leu Val Pro Val Lys Ile Ala Asn His Thr Gly Cys Lys Cys Leu Pro	
	185 190 195	
	ACG GGC CCC CGC CAT CCT TAC TCA ATT ATC AGA AGA TCC ATT CAG ACC	737
	Thr Gly Pro Arg His Pro Tyr Ser Ile Ile Arg Arg Ser Ile Gln Thr	
30	200 205 210	
	CCA GAA GAA GAT GAA TGT CCT CAT TCC AAG AAA CTC TGT CCT ATT GAC	785
	Pro Glu Glu Asp Glu Cys Pro His Ser Lys Lys Leu Cys Pro Ile Asp	
35	215 220 225 230	
	ATG CTG TGG GAT AAC ACC AAA TGT AAA TGT GTT TTG CAA GAC GAG ACT	833
	Met Leu Trp Asp Asn Thr Lys Cys Lys Cys Val Leu Gln Asp Glu Thr	
	235 240 245	
40	CCA CTG CCT GGG ACA GAA GAC CAC TCT TAC CTC CAG GAA CCC ACT CTC	881
	Pro Leu Pro Gly Thr Glu Asp His Ser Tyr Leu Gln Glu Pro Thr Leu	
	250 255 260	
45	TGT GGA CCG CAC ATG ACG TTT GAT GAA GAT CGC TGT GAG TGC GTC TGT	929
	Cys Gly Pro His Met Thr Phe Asp Glu Asp Arg Cys Glu Cys Val Cys	
	265 270 275	
50	AAA GCA CCA TGT CCG GGA GAT CTC ATT CAG CAC CCG GAA AAC TGC AGT	977
	Lys Ala Pro Cys Pro Gly Asp Leu Ile Gln His Pro Glu Asn Cys Ser	
	280 285 290	

EP 0 935 001 A1

	TGC TTT GAG TGC AAA GAA AGT CTG GAG AGC TGC TGC CAA AAG CAC AAG	1025
5	Cys Phe Glu Cys Lys Glu Ser Leu Glu Ser Cys Cys Gln Lys His Lys	
	295 300 305 310	
	ATT TTT CAC CCA GAC ACC TGC AGC TGT GAG GAC AGA TGT CCT TTT CAC	1073
	Ile Phe His Pro Asp Thr Cys Ser Cys Glu Asp Arg Cys Pro Phe His	
10	315 320 325	
	ACC AGA ACA TGT GCA AGT AGA AAG CCA GCC TGT GGA AAG CAC TGG CGC	1121
	Thr Arg Thr Cys Ala Ser Arg Lys Pro Ala Cys Gly Lys His Trp Arg	
	330 335 340	
15	TTT CCA AAG GAG ACA AGG GCC CAG GGA CTC TAC AGC CAG GAG AAC CCT	1169
	Phe Pro Lys Glu Thr Arg Ala Gln Gly Leu Tyr Ser Gln Glu Asn Pro	
	345 350 355	
20	TGATTCAACT TCCTTTCAAG TCCCCCCCATC TCTGTCATTT TAAACAGCTC ACTGCTTGT	1229
	CAAGTTGCTG TCACTGTTGC CCACTACCCCC TGCCCCCCCC CCCCGGCC TCCAGGTGTT	1289
	AGAAAAAGTTG ATTTGACCTA GTGTCATGGT AAAGCCACAT TTCCATGCAA TGGCGGCTAG	1349
	GTGATTCCCC AGTTCACTGA CAAATGACTT GTAGCTTCAA ATGTCTTGCC GCCATCANCA	1409
25	CTCAAAAAGG AAGGGGTCTG AAGAACCCCT TGTTTGATAA ATAAAAACAG GTGCCTGAAA	1469
	CAAATATTA GGTGCCACTC GATTGGTCC CTCGGGCTGG CCAAATTCCA AGGGCAATGC	1529
	TCCTGAATTT ATTGTGCCCTT TTCCTTAATG CGGAATTTC TTTGTTGA TT	1581
30	SEQ ID NO: 25	
	SEQUENCE LENGTH: 1491	
	SEQUENCE TYPE: nucleic acid	
35	STRANDEDNESS: double	
	TOPOLOGY: linear	
	MOLECULE TYPE: cDNA to mRNA	
	ORIGINAL SOURCE:	
40	ORGANISM: rat	
	TISSUE TYPE: lung	
	FEATURE:	
45	NAME/KEY: CDS	
	LOCATION: 270..1247	
	IDENTIFICATION METHOD: E	
	SEQUENCE DESCRIPTION:	
50	GCCACCTCTT GATTATTTGT GCACCGGGAA ACTTTGAAAT AGTTTCATC TCTTTCTCCC	60
	ATACTAAGAT TGTGTGTGGC CGTGGGGAG TCCTTGACTA ACTCAAGTCA TTTCATTGGA	120

EP 0 935 001 A1

	TTTGATTAC AACTGATCAT GTGATATTCTT TTTCCATGTA AAGTTTGGG GCTTCAAACT	180
	TTGCTTCTGG AGAATGCCTT TTGCAACACT TTTCAGTAGC TGCGCTGAAA CAACTGCTTA	240
5	GCCATCAGTG GACATTTGAA ATATTCAAA ATG TAT GGA GAG TGG GCC GCA GTG	293
	Met Tyr Gly Glu Trp Ala Ala Val	
	1 5	
10	AAT ATT CTC ATG ATG TCC TAT GTG TAC CTG GTG CAG GGC TTC AGT ATT	341
	Asn Ile Leu Met Met Ser Tyr Val Tyr Leu Val Gln Gly Phe Ser Ile	
	10 15 20	
15	GAA CAC CGA GCA GTG AAG GAT GTT TCT CTT GAG CGA TCA TCC CGG TCT	389
	Glu His Arg Ala Val Lys Asp Val Ser Leu Glu Arg Ser Ser Arg Ser	
	25 30 35 40	
20	GTG TTG GAA CGT TCT GAA CAA CAG ATC CGC GCG GCT TCT ACT TTG GAA	437
	Val Leu Glu Arg Ser Glu Gln Gln Ile Arg Ala Ala Ser Thr Leu Glu	
	45 50 55	
25	GAG TTG CTG CAA GTC GCA CAC TCT GAG GAC TGG AAG CTG TGG CGG TGC	485
	Glu Leu Leu Gln Val Ala His Ser Glu Asp Trp Lys Leu Trp Arg Cys	
	60 65 70	
30	CGG TTG AAG CTT AAA AGT CTT GCC AAT GTG GAC TCG CGC TCA ACA TCC	533
	Arg Leu Lys Leu Lys Ser Leu Ala Asn Val Asp Ser Arg Ser Thr Ser	
	75 80 85	
35	CAT CGC TCC ACC AGA TTT GCG GCA ACT TTC TAT GAT ACT GAA ACA CTA	581
	His Arg Ser Thr Arg Phe Ala Ala Thr Phe Tyr Asp Thr Glu Thr Leu	
	90 95 100	
40	AAA GTT ATA GAT GAA GAA TGG CAG AGG ACC CAA TGC AGC CCT AGA GAG	629
	Lys Val Ile Asp Glu Glu Trp Gln Arg Thr Gln Cys Ser Pro Arg Glu	
	105 110 115 120	
45	ACA TGC GTA GAA GTC GCC AGT GAG CTG GGG AAG ACA ACC AAC ACA TTT	677
	Thr Cys Val Glu Val Ala Ser Glu Leu Gly Lys Thr Thr Asn Thr Phe	
	125 130 135	
50	TTC AAG CCC CCT TGT GTA AAT GTC TTC CGG TGT GGA GGA TGC TGC AAT	725
	Phe Lys Pro Pro Cys Val Asn Val Phe Arg Cys Gly Gly Cys Cys Asn	
	140 145 150	
	GAA GAG AGC GTG ATG TGT ATG AAC ACA AGC ACC TCC TAC ATC TCC AAA	773
	Glu Glu Ser Val Met Cys Met Asn Thr Ser Thr Ser Tyr Ile Ser Lys	
	155 160 165	
55	CAG CTC TTT GAG ATA TCA GTG CCT CTG ACA TCA GTG CCC GAG TTA GTG	821

EP 0935 001 A1

	Gln Leu Phe Glu Ile Ser Val Pro Leu Thr Ser Val Pro Glu Leu Val			
170	175	180		
5	CCT GTT AAA ATT GCC AAC CAT ACG GGT TGT AAG TGT TTG CCC ACG GGC	869		
	Pro Val Lys Ile Ala Asn His Thr Gly Cys Lys Cys Leu Pro Thr Gly			
185	190	195	200	
10	CCC CGG CAT CCT TAT TCA ATT ATC AGA AGA TCC ATT CAG ATC CCA GAA	917		
	Pro Arg His Pro Tyr Ser Ile Ile Arg Arg Ser Ile Gln Ile Pro Glu			
	205	210	215	
15	GAA GAT CAA TGT CCT CAT TCC AAG AAA CTC TGT CCT GTT GAC ATG CTG	965		
	Glu Asp Gln Cys Pro His Ser Lys Lys Leu Cys Pro Val Asp Met Leu			
	220	225	230	
20	TGG GAT AAC ACC AAA TGT AAA TGT GTT TTA CAA GAT GAG AAT CCA CTG	1013		
	Trp Asp Asn Thr Lys Cys Lys Cys Val Leu Gln Asp Glu Asn Pro Leu			
	235	240	245	
25	CCT GGG ACA GAA GAC CAC TCT TAC CTC CAG GAA CCC GCT CTC TGT GGA	1061		
	Pro Gly Thr Glu Asp His Ser Tyr Leu Gln Glu Pro Ala Leu Cys Gly			
	250	255	260	
	CCA CAC ATG ATG TTT GAT GAA GAT CGC TGC GAG TGT GTC TGT AAA GCA	1109		
	Pro His Met Met Phe Asp Glu Asp Arg Cys Glu Cys Val Cys Lys Ala			
30	265	270	275	280
	CCA TGT CCT GGA GAT CTC ATT CAG CAC CCG GAA AAC TGC AGT TGC TTT	1157		
	Pro Cys Pro Gly Asp Leu Ile Gln His Pro Glu Asn Cys Ser Cys Phe			
	285	290	295	
35	GAA TGC AAA GAA AGT CTG GAA AGC TGT TGC CAA AAG CAC AAG ATG TTT	1205		
	Glu Cys Lys Glu Ser Leu Glu Ser Cys Cys Gln Lys His Lys Met Phe			
	300	305	310	
40	CAC CCT GAC ACC TGC AGA TCA ATG GTC TTT TCA CTG TCC CCT	1247		
	His Pro Asp Thr Cys Arg Ser Met Val Phe Ser Leu Ser Pro			
	315	320	325	
45	TAATTTGGTT TACTGGTGAC ATTTAAAGGA CATACTAACCG TGATTTATTG GGGCTCTTT	1307		
	CTCTCAGGGC CCAAGCACAC TCTTAAAGGA ACACAGACGT TTGGCCTCTA AGAAATACAT	1367		
	GGAAAGTATTA TAGAGTGATG ATTAATTGT CTTCTTGTGTT CAAACAGGGT CTCATGATTA	1427		
50	CAGACCCGTA TTGCCATGCC TGCGTCATG CTATCATGAG CGGAAAAGAA TCACTGGCAT	1487		
	TTAA	1491		

SEQ ID NO: 26

SEQUENCE LENGTH: 20

5 SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

10 MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE DESCRIPTION:

GCTGCGAGTG TGTCTGTAAA

20

15 SEQ ID NO: 27

SEQUENCE LENGTH: 25

20 SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

25 SEQUENCE DESCRIPTION:

GGGTACTGGG CAACAGTGAC AGCAA

25

30

Claims

1. A protein shown by SEQ ID NO: 1 or having the amino acid sequence derived therefrom in which one or more amino acids are substituted, deleted, or added.
2. A protein encoded by a DNA hybridizing with the DNA shown by SEQ ID NO: 2.
3. A DNA encoding the protein of Claim 1.
4. A DNA hybridizing with the DNA shown by SEQ ID NO: 2.
5. A vector containing the DNA of Claim 3 or 4.
6. A transformant carrying the vector of Claim 5.
7. A method of producing the protein of Claim 1 or 2, which comprises culturing the transformant of Claim 6.
8. An antibody binding to the protein of Claim 1 or 2.
9. A method of screening a compound binding to the protein of Claim 1 or 2, which comprises a step of detecting the activity of the protein of Claim 1 or 2 to bind to a test sample.
10. A compound binding to the protein of Claim 1 or 2, wherein the compound have been isolated by the method of Claim 9.

Fig. 1

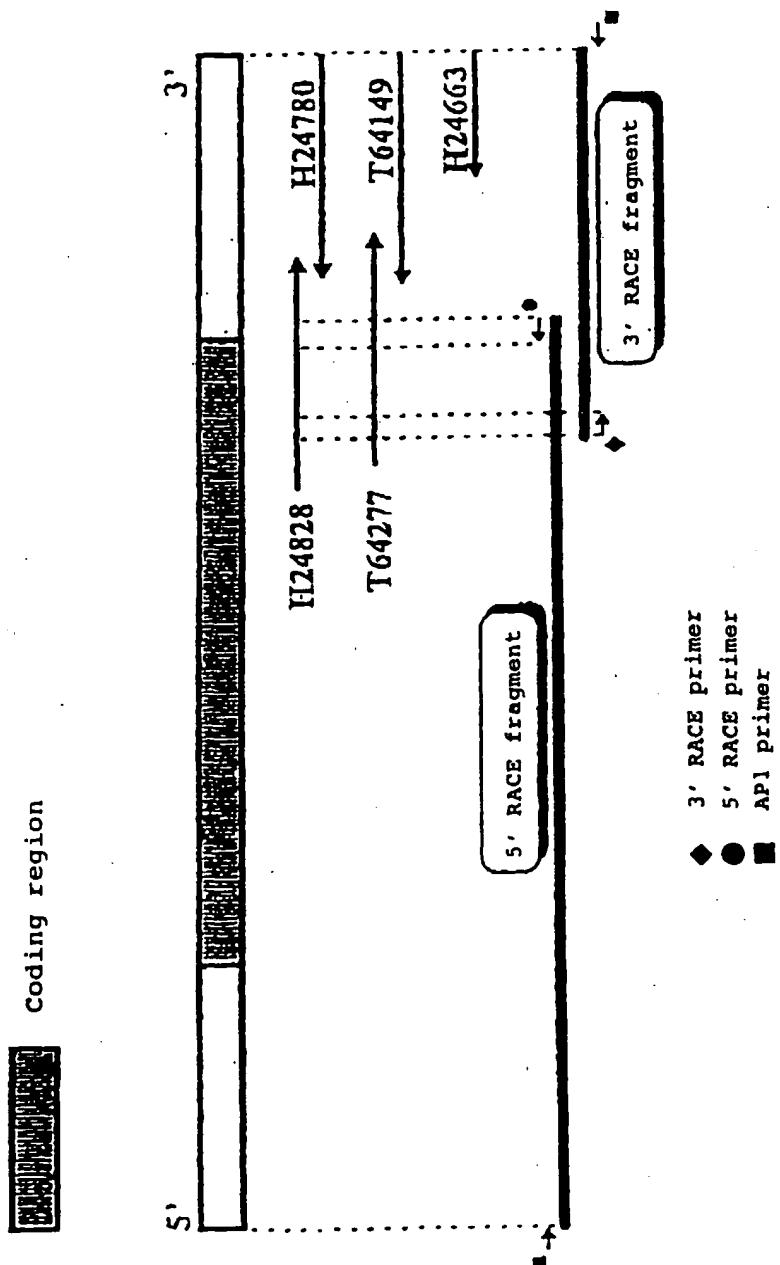


Fig. 2

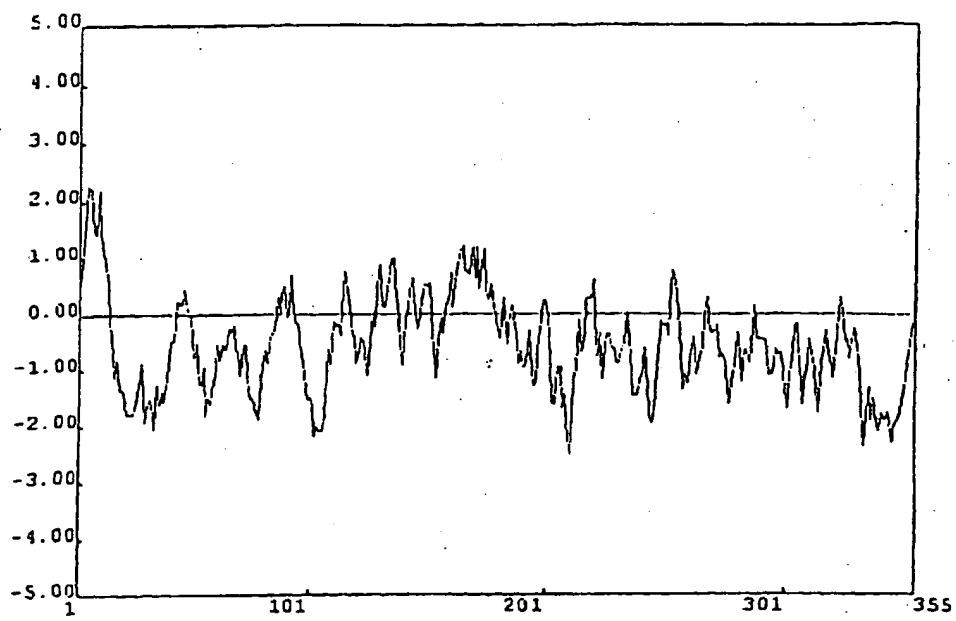
HSVEGFCC*	MHLLGFFSYA CSLLAAALLP GPREAPAAAA AFESGLDLSD AEPODAGEATA	50
H24828	-----	50
HSVEGFCC	YASKDLEEQL RSVSSVDELM TVLYPEYWKM YKCQLRKGGW QHNREQANLN	100
H24828	-----	100
HSVEGFCC	SRTEETIKFA AAHYNTEILK SIDNEWRKTQ CMPREVCIDV GKEFGVATNT	150
H24828	-----	150
HSVEGFCC	FFKPPCVSYY RCGGCCNSEG LQCHMTSTSY LSCTLFEITY PLSQGPKPVT	200
H24828	-----	200
HSVEGFCC	ISFANHTSCR CMSKLDVYRQ VHSIIRSLP ATLPQCQAAN KTCPTNYMWN	250
H24828	-----	250
HSVEGFCC	NHICRCLAEQ DFMFSSDAGD DSTDGFDIC GPNKELDEET CQCVCRAGLR	300
H24828	-----	300
HSVEGFCC	PASCCPHKEL DRNSCOCVCK NKLFPSQCGA NREFDENTCQ CVCKRTOPRN	350
H24828	PAEOPHMMF DEDRSCOCVCK TPCPKDLIQH PKNCSCECK ESLETCCQKH	350
HSVEGFCC	OPLNPGKAAC OCTESPQKCL LKGKKFHQT ESCYRRPCTN ROKAC-EPGF	400
H24828	KLFHEDTSS E -----DR CPFHTRPCAS GKTACAKHCR	400
HSVEGFCC	SYSSEVRCV PSYVWRPOMS	450
H24828	FPKEKRAAQG CHSRHNG	450
*HSVEGFCC:		human VEGF-C

Fig. 3

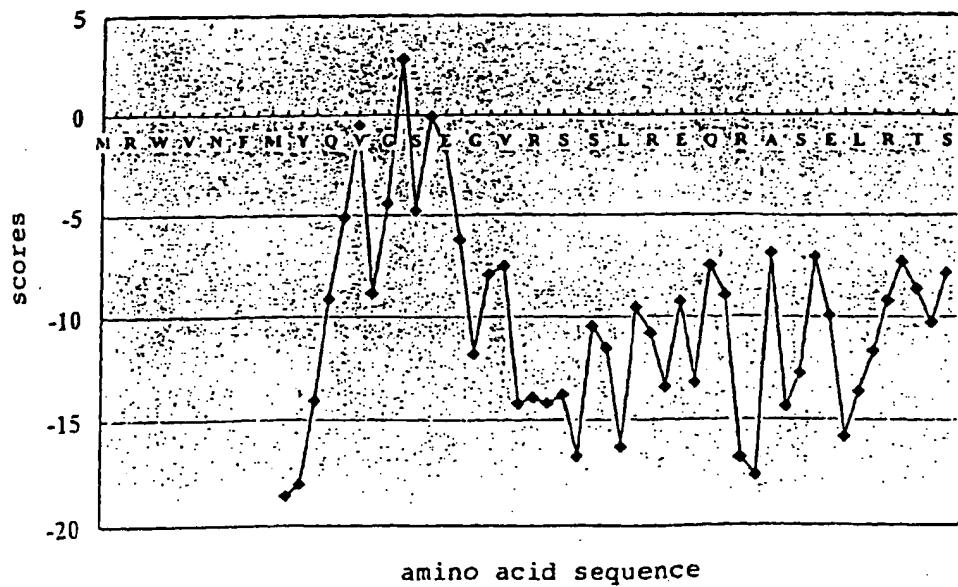
HSVEGF-D	HYRFVVVVNV FMMILYVOLVO GSSNEHGPVK -----RSSQ	50
HSVEGF-C	XHLIGFFESVA CSLAALLP GPREAPAAAA AFESGLLSD AEPDAGEATA	50
HSPDFG-A	ARTIACLLL GCGYIAHVLA EEAEIFPREVI ERLAR-----SQ	50
HSPDFG-B	ENRCWALFLS LCCYIURVSA EGDPPIPEELY EHLSD-----HS	50
HSPIGF2	MPVMRLFPCF LOLAGLAGLP AVPPQWALS AGNGS-----Y	50
HSVEGF	SNFILSWVHW SLALILYIH AKWSQAAPMA EGGO-----	50
HSVEGF-B	YSPULRR--- --LLAALLO LAPAQAPVSQ PDAPG-----	50
HSVEGF-D	STLERSEQQI RAASSLEELL RITHSEDWKL WRGRRLRKSFS TSMDSRSASH	100
HSVEGF-C	YACKDLEFQL RSVSSVDELM TVDYPEYWKKM YKCOLRKGGW QHNREQANLN	100
HSPDFG-A	IHRISIROLRL LEIDSYGSED S-----DTSLRA	100
HSPDFG-B	IRSFDDLCRL LHGDPGEDG AE-----DLNMTR	100
HSPIGF2	-----	100
HSVEGF	-----	100
HSVEGF-B	-----	100
HSVEGF-D	RST---RFA ATFYDIETLK VIDEEWQRTQ SPREITCDEV ASBLGKSINT	150
HSVEGF-C	SRTEETIKFA DAHYNTTILK SIONEWRKTQ CMPREVCIDV GKFGVAVINT	150
HSPDFG-A	HGVHATKHVP EKRPLPIRRR RSLIEEAVPAV KPTIVIYEI PRSQVDPUSA	150
HSPDFG-B	SHSGGELESI ARGRRSIGSL TIAEPMIAE KTTSTEVEFI SRLLIDRUNA	150
HSPIGF2	-----SEVEVVP FOEV-WGRSY KRALFRLLDV VSGYPSEVEH	150
HSVEGF	-----NHHHEVVK FMBV-YQRSY SHIETLVLIDI FOBYPDEIEY	150
HSVEGF-B	-----HQKVVVS WIDY-YTAT COPREVVVPL TVELMGTVAK	150
HSVEGF-D	FF--KPPCVN FRCGGCCHE BSICGMNST SYISKTLFEI -SPLTSVPE	200
HSVEGF-C	FF--KPPCVS YRCGGCCRS EGQDMNST SYLSKTLFEI -TPLSQGPK	200
HSPDFG-A	NFLINPPCVIE SKRCGCGCT SSYKQOPSRV HHRSVXVAKY EYVGKKPKLK	200
HSPDFG-B	NFLVWPPCVIE DORISGCCMN RNYQDRPTQV OLRPVQVRK EIVVKKPIFK	200
HSPIGF2	MF--SFSCVS LLRGCGCGGD ENDHGWFVET ANVTMOLLKI ---BSGDRPS	200
HSVEGF	IF--KFCSCV P IMRCGGCCND EGIEEVPTEE SNITMOIMRI ---PHQGQH	200
HSVEGF-B	QL--VSCCVT QRCGGCCCPD DGUEEVPIGO HQVRMQLMLI ---CYPSSQ-	200
HSVEGF-D	LPPVKYANHT GOKLPLT--A PRHPYSIIRP SIOIPEEDFC SHSALLCPID	250
HSVEGF-C	PHTISFANHT SORCHSKLDV YROVHSIIIRE S-SPATLPOC QAAAHNTCPIN	250
HSPDFG-A	ELOVRLLEEHL EAGATTSLN PDYREEDTG P-RESGKSK E--DRLKPI-	250
HSPDFG-B	KATVTLLEDHL AKESET-VAA ARPVTVRSPGC S-QEORA-----	250
HSPIGF2	YVELTFSOHV RCEERPF--- LREKMKPERF R-PKGGRGPER E--EMORPT-	250
HSVEGF	IGEMSFLOH KGEGR-KKD RAROEKKSVF G-KGKGQWFK E--XWSRYK-	250
HSVEGF-B	LGEMLSEEH QLESPKKKD SA-----	250
HSVEGF-D	MWDNSNKCKC VIQEE-NPLA GTEDSHLQE -----	300
HSVEGF-C	YMWNNHHICRG LAOEDMFSS DAGCSTDGF HDICGPNKEL EEECOVDR	300
HSPDFG-A	-----	300
HSPDFG-B	-----	300
HSPIGF2	-----DCBLCGDA VPVR	300
HSVEGF	-----BWSVYY GRCCLAFWS LPGP-PCGPC SERRKLFVQ DFOTCKOSCK	300
HSVEGF-B	-----VKPESPRPLC PRCQSHORP DFRECRCRGR	300
HSVEGF-D	-----PALCQP HMMFDEDRC EVCATPCPKD LIQHPKNCSF FECKESL-E	350
HSVEGF-C	AGLRPASCP EKEIDRNSEQ EVCBNKLFPS QCGANREFDE NTQQCVCKR	350
HSPDFG-A	-----	350
HSPDFG-B	VRVRRPPKSK ERKFKHTHDK TALEETUGA	350
HSPIGF2	-----	350
HSVEGF	N-TDSRCKAR OLEINERTCR GDKPRR	350
HSVEGF-B	RRSFLRCQGR GLEINPDTCR ERLKLR	350
HSVEGF-D	CCQKHKLFHF DTCSCE----- DRCPFHT RPCASGKTAQ	400
HSVEGF-C	CPRNQPL-NP GKACACTES PQKCLLKGKK FHHQTQSCYR RFCTNRQKAC	400
HSPDFG-A	-----	400
HSPDFG-B	-----	400
HSPIGF2	-----	400
HSVEGF	-----	400
HSVEGF-B	-----	400
HSVEGF-D	AKHCRFP4EK RAAOGPHSRK NF.	450
HSVEGF-C	-EPGFSYSEE VRCCVSYWA RQOMS	450
HSPDFG-A	-----	450
HSPDFG-B	-----	450
HSPIGF2	-----	450
HSVEGF	-----	450
HSVEGF-B	-----	450

Fig. 4

a) Hydrophobicity



b) Prediction of the human VEGF-D signal peptide



INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP97/02456

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl ⁶ C12N15/18, C12N15/63, C12P21/02, C07K14/485, C07K16/22, G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int. Cl⁶ C12N15/18, C12N15/63, C12P21/02, C07K14/485, C07K16/22, G01N33/50

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPI, WPI/L, BIOSIS PREVIEWS, CAS ONLINE, GENETYX-MAC/CD

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	Yamada, Y. et al. "Molecular cloning of a novel vascular endothelial growth factor, VEGF-D." Genomics (1997, Jun.), Vol. 42, No. 3, p. 483-488	1 - 10
X	Vladimir, J. et al. "A novel vascular endothelial growth factor, VEGF-C, (VEGFR-2) receptor tyrosine kinases" EMBO J. (1996, Jan.) Vol. 15, No. 2, p. 290-298	1 - 2
X	Vladimir, J. et al. "A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4(VEGFR-3) and KDR(VEGFR-2) receptor tyrosine kinases" EMBO J. (1996, Jan.) Vol. 15, No. 7, p. 1751	1 - 2
PX	Maurizio, O. et al. "Identification of a c-fos-induced gene that is related to the platelet-derived growth factor/vascular endothelial growth factor family" Proc. Natl. Acad. Sci. USA (1996, Oct.) Vol. 93, p. 11675-11680	1 - 2

 Further documents are listed in the continuation of Box C. See patent family annex.

- * Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search October 7, 1997 (07. 10. 97)	Date of mailing of the international search report October 21, 1997 (21. 10. 97)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT		International application No. PCT/JP97/02456
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Georg. B. et al. "Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation" Development (1992) Vol. 114, p. 521-532	1 - 10
X	David, T.S. et al. "The mouse gene for vascular endothelial growth factor" J. Biol. Chem. (1996, Feb.) Vol. 271, No. 7, p. 3877-3883	1 - 10
X	Kevin, P.C. et al. "Vascular endothelial growth factor" J. Biol. Chem. (1992) Vol. 267, No. 23, p. 16317-16322	1 - 10
X	Greg, C. et al. "Amino acid and cDNA sequences of a vascular endothelial cell mitogen that is homologous to platelet-derived growth factor" Proc. Natl. Acad. Sci. USA (1990) Vol. 87, p. 2628-2632	1 - 10
X	Edmund, T. et al. "The human gene for vascular endothelial growth factor" J. Biol. Chem. (1991) Vol. 266, No. 18, p. 11947-11954	1 - 10

Form PCT/ISA/210 (continuation of second sheet) (July 1992).

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP97/02456

Disclosure other than written disclosures

1. The GenBank Database (Rel. 100) on GENETYX, Accession No. D89628, Yoshiki Yamada, Chugai Research Institute for Molecular Medicine. (29-Nov-1996)
2. The GenBank Database (Rel. 100) on GENETYX, Accession No. T64277, Hillier, L. et al. (1995)